

WHAT IS CLAIMED IS:

1. A method of identifying an agent that alters the level of surface expression of an integral membrane protein in a mammalian cell, said method comprising:

a) preparing a first medium containing mammalian cells that express a first mutant form of said membrane protein, wherein said first mutant form is expressed on the surface of said cells at a level less than a wild-type form of said protein;

b) adding to said first medium containing mammalian cells an effective amount of a candidate agent;

c) incubating said cells in the presence of said candidate agent for a sufficient period of time;

d) adding to said first medium containing mammalian cells an effective amount of at least one antibody which binds to at least one extracellular epitope of said mutant form of said membrane protein; and

e) determining the level of binding of said at least one antibody to said extracellular epitope following incubation with said candidate agent, wherein a change in said level of binding relative to control indicates that said candidate agent alters the level of surface expression of said mutant form of said membrane protein.

2. The method according to claim 1, further comprising the steps of:

f) preparing a second medium containing mammalian cells that express a second mutant form of said membrane protein, said second mutant form is different from said first mutant form and is expressed on the surface of said mammalian cells at a level lower than that of a wild-type form of said membrane protein;

g) adding to said second medium containing mammalian cells an effective amount of said candidate agent;

h) incubating said cells in the presence of said candidate agent for a sufficient period of time;

i) adding to said second medium containing mammalian cells an effective amount of at least one antibody which binds to at least one extracellular epitope of said second mutant form of said membrane protein; and

j) determining the level of binding of said at least one antibody to said extracellular epitope of said second mutant form of said membrane protein following incubation with said candidate agent,

wherein a change in said level of binding relative to control indicates that said candidate agent alters the level of surface expression of said second mutant form of said membrane protein.

3. The method according to claim 1 or 2, wherein step (d) comprises adding an effective amount of at least one primary antibody and an effective amount of at least one

secondary antibody, wherein said primary antibody binds to at least one extracellular epitope of said first mutant form of said membrane protein and said secondary antibody binds to said first antibody.

4. The method according to claim 1 or 2, wherein said level of binding is measured by fluorescence, luminescence, radioactivity, absorbance or a combination of two or more thereof.

5. The method according to claim 1 or 2, wherein said integral membrane protein is a membrane ion channel.

6. The method according to claim 5, wherein said membrane ion channel is a sodium channel, a potassium channel, a calcium channel or a chloride channel.

7. The method according to claim 1 or 2, wherein said at least one extracellular epitope comprises a wild-type epitope.

8. The method according to claim 1 or 2, wherein said at least one extracellular epitope contains a tag.

9. The method according to claim 8, wherein said extracellular tag replaces at least a portion of an extracellular domain of said integral membrane protein.

10. The method according to claim 8, wherein said extracellular tag is inserted in an extracellular domain of said membrane protein.

11. The method according to claim 8, wherein said extracellular tag comprises a hemagglutinin (HA) tag.

12. The method according to claim 3, wherein said primary antibody and/or said secondary antibody is coupled to an enzyme.

13. The method according to claim 12, wherein said enzyme is selected from the group consisting of peroxidases, luciferases, alkaline phosphatases, glucose oxidases, beta-galactosidases and mixtures of two or more thereof.

14. The method according to claim 1 or 2, wherein said first mutant form comprises an amino acid sequence which differs in at least one amino acid residue from the amino acid sequence of a wild-type form of said membrane protein.

15. The method according to claim 2, wherein said second mutant form comprises an amino acid sequence which differs in at least one amino acid residue from the amino acid sequence of a wild-type form of said membrane protein.

16. The method according to claim 1 or 2, wherein said first mutant form is a trafficking-deficient mutant.

17. The method according to claim 2, wherein said second mutant form is a trafficking-deficient mutant.

18. The method according to claim 1 or 2, wherein said membrane protein is a potassium ion channel.

19. The method according to claim 18, wherein said potassium ion channel is hERG.

20. The method according to claim 19, wherein said first mutant form of hERG is G601S.

21. The method according to claim 19, wherein said first mutant form of hERG is N470D.

22. The method according to claim 19, wherein said second mutant form of hERG is G601S/F656C.

23. The method according to claim 19, wherein said second mutant form of hERG is N470D/F656C.

24. A method for preventing or treating cardiac arrhythmia comprising administering to a mammal in need thereof an effective amount of an active agent, wherein said active agent increases the level of surface expression of a first mutant form of hERG in a mammalian cell and does not increase the level of surface of a second mutant form of hERG in a mammalian cell as determined by the method comprising:

a) preparing a first medium containing mammalian cells that express said first mutant form of hERG, wherein said first mutant form is expressed on the surface of said mammalian cells at a level lower than that of a wild-type form of hERG;

b) adding to said first medium an effective amount of said active agent;

c) incubating said cells in the presence of said active agent for a sufficient period of time; and

d) adding to said first medium containing mammalian cells an effective amount of at least one antibody which binds to at least one extracellular epitope of said mutant form of hERG;

- e) determining the level of binding of said at least one antibody to said extracellular epitope;
- f) preparing a second medium containing mammalian cells that express a second mutant form of hERG, wherein said second mutant form is different from said first mutant form and is expressed on the surface of said mammalian cells at a level lower than that of a wild-type form of hERG;
- g) adding to said second medium containing mammalian cells an effective amount of said active agent;
- h) incubating said cells in the presence of said active agent for a sufficient period of time;
- i) adding to said second medium containing mammalian cells an effective amount of at least one antibody which binds to at least one extracellular epitope of said second mutant form of hERG; and
- j) determining the level of binding of said at least one antibody to said extracellular epitope of said second mutant form of hERG.

25. The method according to claim 24, wherein said active agent is vanoxerine or a pharmaceutically acceptable salt thereof.

26. The method according to claim 24, wherein step d) comprises adding an effective amount of at least one primary antibody and an effective amount of at least one secondary

antibody, wherein said primary antibody binds to at least one extracellular epitope of said first mutant form of hERG and said secondary antibody binds to said first antibody.

27. The method according to claim 24, wherein said level of binding is measured by fluorescence, luminescence, radioactivity, absorbance or a combination of two or more thereof.

28. The method according to claim 24, wherein said at least one extracellular epitope contains a tag.

29. The method according to claim 28, wherein said extracellular tag replaces at least a portion of an extracellular domain of hERG.

30. The method according to claim 28, wherein said extracellular tag is inserted in an extracellular domain of hERG.

31. The method according to claim 28, wherein said extracellular tag comprises a hemagglutinin (HA) tag.

32. The method according to claim 26, wherein said primary antibody and/or said secondary antibody is coupled to an enzyme.

33. The method according to claim 32, wherein said enzyme is selected from the group consisting of peroxidases, luciferases, alkaline phosphatases, glucose oxidases, beta-galactosidases and mixtures of two or more thereof.

34. The method according to claim 24, wherein said first mutant form is a trafficking-deficient mutant.

35. The method according to claim 24, wherein said first mutant form of hERG is G601S.

36. The method according to claim 24, wherein said first mutant form of hERG is N470D.

37. The method according to claim 24, wherein said second mutant form of hERG is G601S/F656C.

38. The method according to claim 24, wherein said second mutant form of hERG is N470D/F656C.

39. The method according to claim 24, wherein step i) comprises adding an effective amount of at least one primary antibody and an effective amount of at least one secondary

antibody, wherein said primary antibody binds to at least one extracellular epitope of said second mutant form of hERG and said secondary antibody binds to said first antibody.